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Gymnopilus purpureosquamulosus Høil. (Agaricales, Basidiomycota): a new distributional record from India

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Abstract: *Gymnopilus purpureosquamulosus* Høil (*Strophariaceae*) is reported for first time from India. A comprehensive description, a photograph, and comparisons with morphologically similar and phylogenetically related species are provided.

Key words: new record; nrDNA; phylogenetic analysis; West Bengal; wood-inhabiting fungi

The genus *Gymnopilus* P. Karst. is comprised of approximately 200 species of wood-inhabiting fungi that are worldwide in distribution (Holec 2005; Kirk et al. 2008). Morphologically the genus is well characterized by a combination of features that include a brightly coloured fruitbody with adnexed to decurrent lamellae, the presence of a membranous veil, rust-brown coloured basidiospores that are dextrinoid in nature with verrucose to rugulose ornamentation, well-developed cheilocystidia that are fusiform or lageniform with their apices capitate to subcapitate or obtuse, and the presence of clamp connections in all the tissues (Holec 2005; Guzmán-Dávalos et al. 2008; Kaur et al. 2015).

Presently, the genus is included within the family Strophariaceae as proposed by Kühner (1980). However, Singer (1986) placed the genus in the family Cortinariaceae primarily due to the ferruginous spore-print and basidiospores that possess a compound wall ornamented with warts.

A literature review finds that about 25 species of *Gymnopilus* have been reported from India so far (Kulkarni 1990; Thomas et al. 2003; Ministry of Environment & Forests 2011; Farook et al. 2013; Kumar et al. 2014; Kaur et al. 2015). Only four species are known from the state of West Bengal: *Gymnopilus dilepis* (Berk. & Broome) Singer; *G. chrysimyces* (Berk.) Manjula; *G. chrysites* (Berk.) Singer; and *G. hybridus* (Bull.) Maire (Pradhan et al. 2013; Manjula 1983). The present study adds *G. purpureosquamulosus* Høil. to the list of Indian mycoflora.

During a field trip in August 2015 for the purpose of macrofungal inventory, a specimen was collected from Katlia, Howrah, West Bengal, India. The morphological and ecological (habit and habitat) characters of this specimen were noted in the field. Colour codes and terms follow the Kornerup & Wanscher (1978). Dried basidiomata were sectioned by hand, mounted in a mixture of 5% KOH, Congo red, and Melzer's reagent and microscopic features viewed using a Carl Zeiss AX10 Imager A1 phase contrast microscope. Measurements of basidiospores were noted from 30 randomly chosen basidiospores from each of the collected basidiomata (n = 5); values in parentheses indicate minimum or maximum measured values and Q value of the basidiospore denotes length/width ratio of the spores excluding ornamentation (Acharya et al. 2015). A voucher specimen was preserved using the protocol described by Pradhan et al. (2015) and deposited in the Calcutta University Herbarium (CUH).

Genomic DNA was extracted from the dried fruitbody following Dutta et al. (2015). PCR amplification of the nuclear ribosomal internal transcribed spacer sequence (nrITS) region was performed using fungal universal primers pair ITS1 and ITS4 (White et al. 1990) on an Applied Biosystems 2720 automated thermal cycler using the thermal profile as described by Dutta et al. (2015). PCR products were then purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany) and were subjected to automated DNA sequencing on ABI3730xl DNA Analyzer (Applied Biosystems, USA) using the same primer pairs used for the amplification of rDNA ITS region.

The newly generated sequence of *G. purpureosquamulosus* was then edited using CodonCode Aligner software (CodonCode Corporation, Dedham, Massachusetts) and used for a BLAST search in the NCBI database. Altogether 31 nrDNA ITS sequences of *Gymnopilus* representing 20 species were chosen for the phylogenetic analyses based on the BLAST search and the previous study of Guzmán-Dávalos et al. (2008). *Dermocybe sanguinea* (Wulfen) Wünsche [currently *Cortinarius sanguineus* (Wulfen) Fr.], *Galerina clavata* (Velen.) Kühner, and *Psilocybe cubensis* (Earle) Singer were selected as out-group taxa for rooting purpose following Guzmán-Dávalos et al. (2003).

All these sequences were then aligned with ClustalX (Thompson et al. 1997) using default settings. The alignment was then imported into MEGA v. 6.0 (Tamura et al. 2013) for additional manual adjustments. The ends of the alignment were trimmed to create a data set of 658 bp in length. The appropriate model of evolution for phylogenetic analysis was determined using jModeltest 2.1.6 v20140903 (Darriba et al. 2012) in the CIPRES web portal (Miller et al. 2009). Based on the Bayesian information criterion (BIC), the GTR+I+G (6808.186355) model was selected for the ITS data.

Maximum likelihood (ML) analyses was performed with RAxML 8.2.9 (Stamatakis 2014) on the CIPRES NSF XSEDE resource, using the parameters specified by jModeltest 2.1.6 v20140903 with bootstrap statistics calculated from 1000 bootstrap replicates. Bayesian phylogenetic analyses were carried out using Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) methods with MrBayes v. 3.2.2 (Ronquist et al. 2012), under a GTR+I+G model. For a given data set, the General time reversible (GTR) model was employed with gamma-distributed substitution rates. Markov chains were run for one million generations, saving a tree every 100th generation. Default settings in MrBayes were used for the incremental heating scheme for the chains (3 heated and 1 cold chain), unconstrained branch length (unconstrained: exponential (10.0)), and uninformative topology (uniform) priors. MrBayes was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates of the posterior probabilities (PPs) of the groups. Bayesian posterior probabilities values ≥ 0.95 are shown in the resulting trees as thickened line.

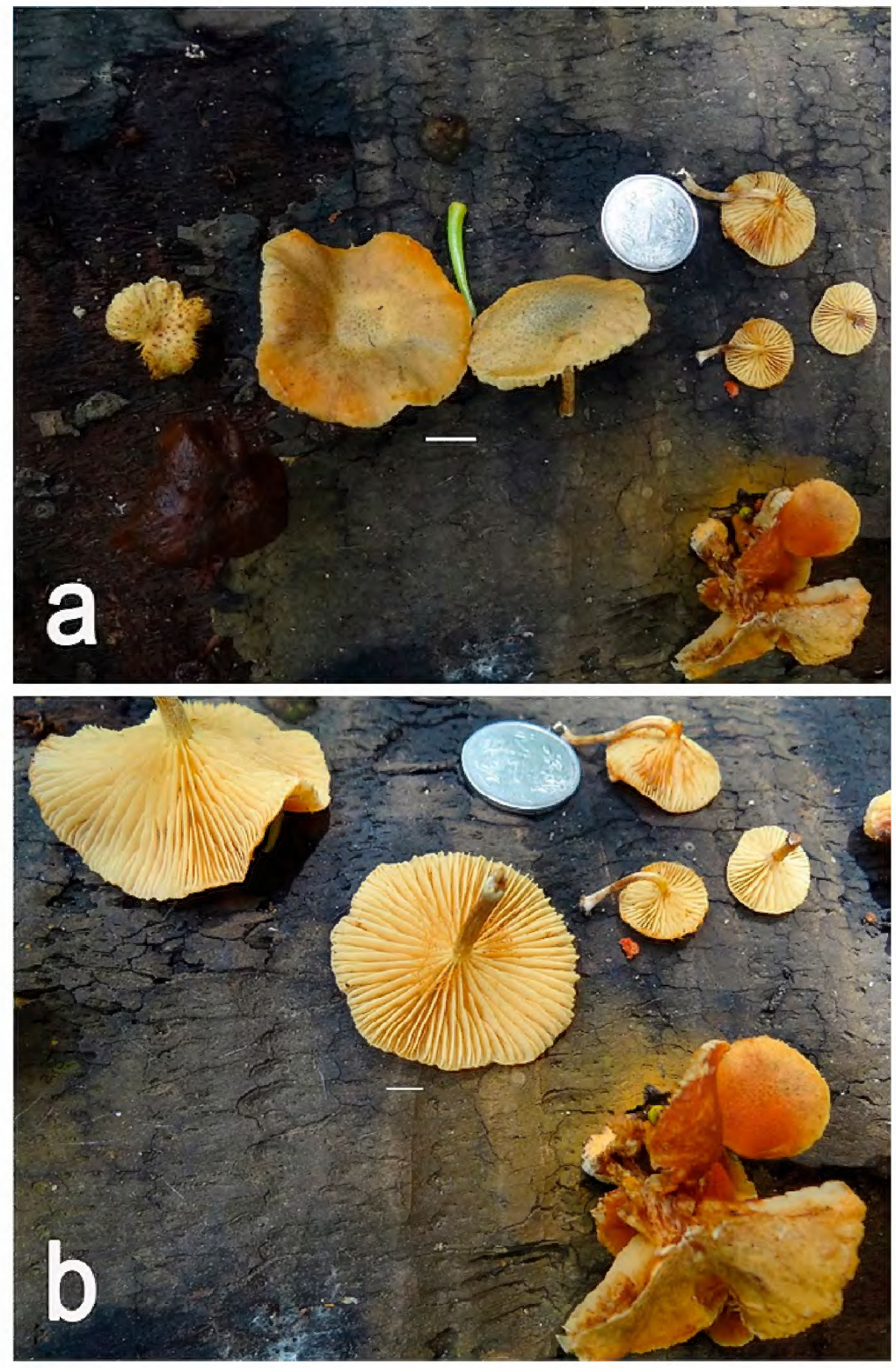

Figure 1. *Gymnopilus purpureosquaulosus* (CUH AM252), **a.** Field photograph of the basidiomata showing pileus surface. **b.** Mature basidiomata showing lamellae characteristics. Scale bar = 10 mm.

Gymnopilus purpureosquamulosus Høil.
(Høiland 1998: 82)
Figures 1, 2

Pileus 9–65 mm in diameter, convex, broadly convex to concave with slightly central depression when young, becoming plano-convex to plane at maturity with a slight central depression, moist, translucent striate, wavy at mature, orange yellow (4A8), light orange (5A4), greyish orange (5B3) to brownish orange (5C5-C6; 6C3), orange grey (6B2) or reddish (10A2), turns dark brown (6F4) to gray (3F1) with KOH and $FeSO_4$, unchanging with NH_4OH and phenol; surface squammules, dense and erect to suberect towards centre, scattered and appressed towards margin, tiny, brown purple to purple; context ca. 3 mm thick, light yellow (3A5). Lamellae adnate to subdecurrent, up to 2 mm wide, subdistant with 3–4 series of lamellulae, greyish orange (5B4; 6B3-B4), yellowish brown (5E8), light orange (6A4) to often dark brown (6F4), turns grey (4E1) with KOH and $FeSO_4$, edge even to slightly wavy, concolourous. Stipe 21–60 × 5–9 mm, central, cylindrical to subcylindrical, surface fibrillose, grey (5E1), brown (7E5) to reddish brown (8D4-E4), no colour change on brushing, turns dark brown (6F4) with KOH, brown (6E4) with $FeSO_4$; context solid, yellowish white (1A2) to pale yellow (2A3). Veil membranous, yellow to brown yellow. Spore print brown to rusty brown.

Basidiospores 7–8(–9.5) × 4–5.5(–6.5) µm, *Q* = 1.5–1.8, ellipsoid to oblong with subacute apex, thick-walled, verrucose, warts medium, germ pore absent, with a slight suprahilar depression, yellowish brown with KOH, dextrinoid. Basidia 18–26 × 7–9.5 µm, clavate to subclavate, hyaline, oil granules present when viewed with KOH, basal cell irregular in shape, with well-developed clamp connections, 4-spored; sterigmata 3.5–4.5 × 1–1.5 µm long. Pleurocystidia not observed. Cheilocystidia 21.5–25 × 4.5–7.5 µm, subclavate, cylindrical to fusiform or lageniform with obtuse apex, hyaline, thin-walled. Hymenophoral trama hyphae subparallel, cylindrical, hyaline. Subhymenium consists of elongated cellular elements. Pileipellis a cutis type, composed of 7–10.5 µm broad, hyaline, thin-walled hyphae, oil granule present when viewed with KOH, clamp-connections present. Pileocystidia 40–54 × 9–11 µm, subclavate to cylindrical with mucronate to obtuse apex. Stipitipellis hyphae 5.5–9 µm in diameter, hyaline,

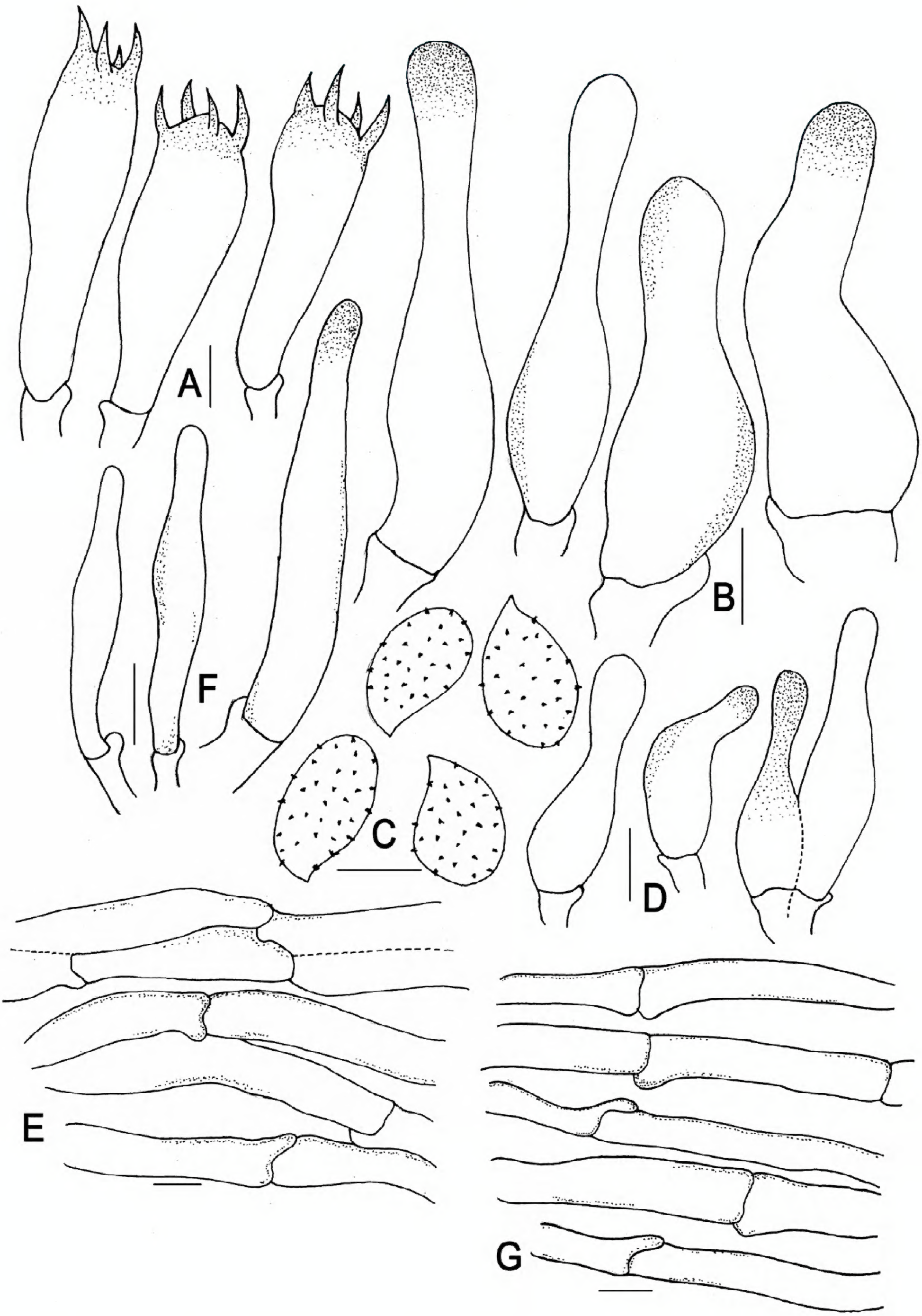

Figure 2. *Gymnopilus purpureosquaulosus* (CUH AM252) **A.** Basidium. **B.** Cheilocystidia. **C.** Basidiospores. **D.** Caulocystidia. **E.** Hyphal arrangement of Pileipellis. **F.** Pileocystidia. **G.** Hyphal arrangement of stipitipellis. Scale bars: A, B = 5 µm; C–G = 10 µm.

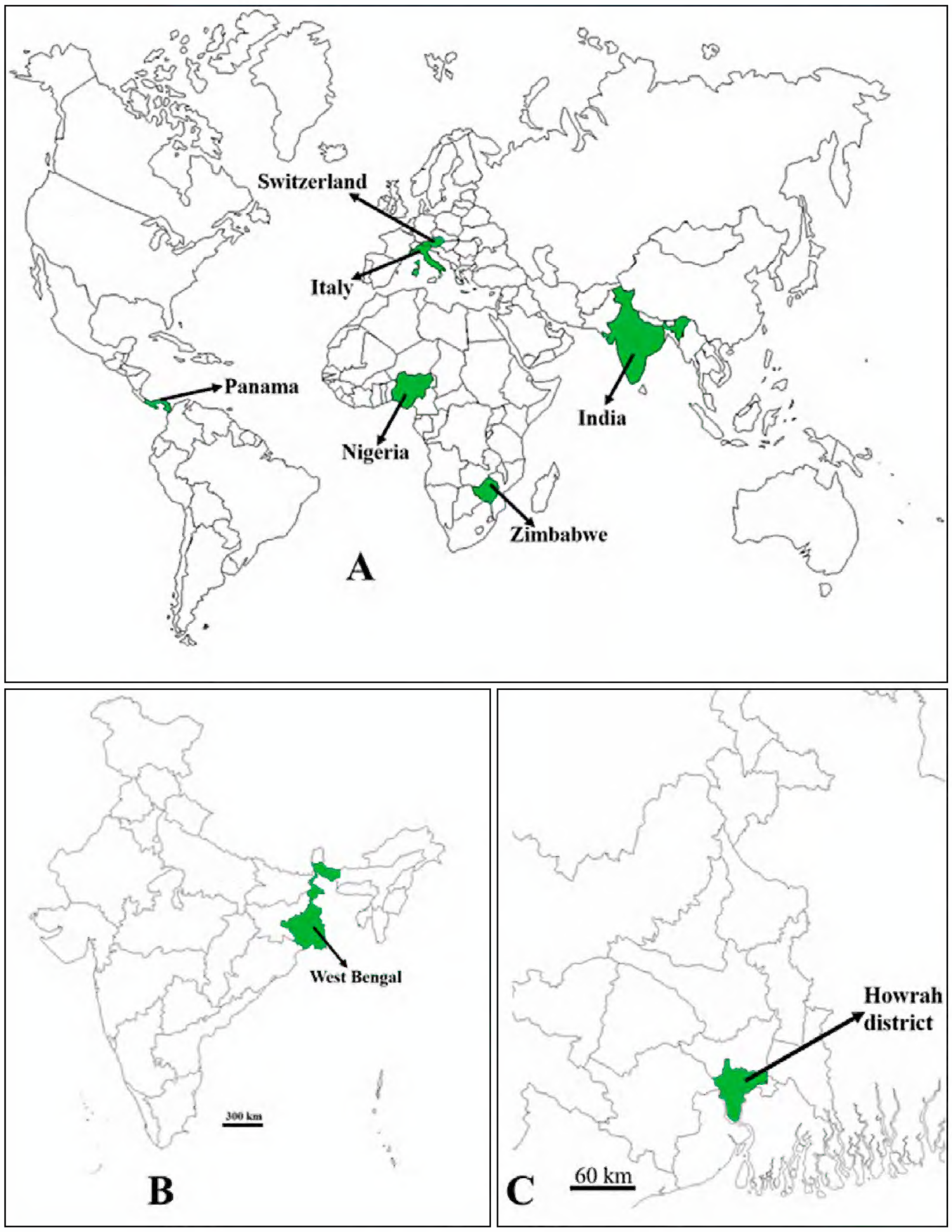

Figure 3. *Gymnopilus purpureosquamulosus*. **A.** World distribution, countries marked with green. **B.** India map, showing the state in green from where the specimen was collected. **C.** Map of West Bengal showing the collection place.

thin-walled. Stipe trama hyphae parallel, cylindrical, hyaline, thin-walled. Caulocystidia 36–43 × 5.5–6.5 µm in diameter, similar in shape to cheilocystidia.

Habit and habitat: Gregarious to scattered, on dead and decomposed wood.

Distribution: Zimbabwe (Høiland 1998), Italy, Nigeria, Panama, Switzerland (Guzmen-Dávalos et al. 2008), Brazil (Neves et al. 2013), and now India (Figure 3).

Specimen examined: India: West Bengal, Howrah, Katlia, 22°37′09″ N, 088°15′12″ E, 29 August 2015, Tulika Saha, TULIKA-02 (CUH AM252).

Diagnostic features of *Gymnopilus purpureosquamulosus* includes a medium-sized to large pileus coloured whitish yellow, dull yellow to orange yellow or golden ochre to orange grey that turns brown with KOH, presence of purplish squammules on the pileus surface, adnate to subdecurrent, yellowish brown to light orange or greyish lamellae that possess 3–4 series of lamellulae, a stipe coloured grey to reddish brown that turns brown with KOH, presence of membranous veil on the stipe, ellipsoid to oblong basidiospores measuring 7–9.5 × 4–6.5 µm (Q = 1.5–1.8), presence of inflated elements in the subhymenium, cutis type of pileipellis, and presence of pileocystidia (Høiland 1998; Guzmán-Dávalos et al. 2008).

Gymnopilus purpureosquamulosus was originally described based on the collection made from Zimbabwe (Høiland 1998). Later, Guzmán-Dávalos et al. (2008) reported the same species from Italy, Nigeria, Panama, and Switzerland.

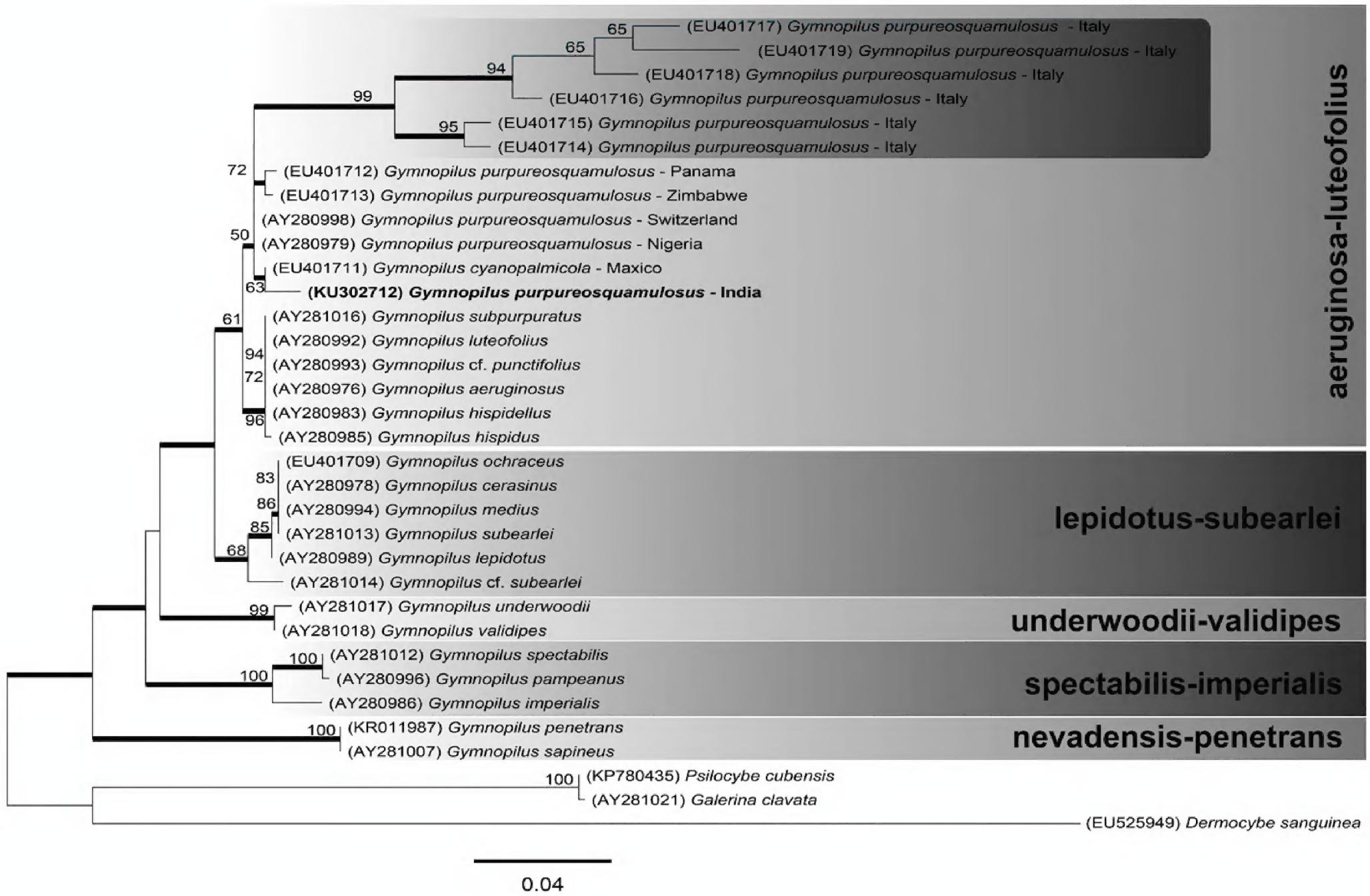

Figure 4. Maximum likelihood tree (-lnL = 3160.453779) generated using a GTR+I+G model of nucleotide evolution for the ITS sequence data. Numbers above the branch lengths refer to ML bootstrap percentages (≥50 %). Bayesian posterior probabilities (PP) ≥0.95 are indicated as black coloured thickened lines and the scale bar represents the expected changes per site. The newly generated sequence of *Gymnopilus purpureosquaulosus* is placed in bold font to highlight its phylogenetic position in the tree. Demarcation of the clades follows GUZMÁN-DÁVALOS et al. (2003).

The morphological features of our Indian collection nicely match with that of the type description made by HØILAND (1998), but concordant with GUZMÁN-DÁVALOS et al. (2008), the squammules on the pileus centre was erect to suberect and appressed towards margin.

The newly generated sequence was deposited in GenBank (http://www.ncbi.nlm.nih.gov) with the accession number KU302712. Bayesian analyses reached a standard deviation of split frequencies of 0.003 after one million generations. In the phylogenetic tree (Figure 4), 20 included species of *Gymnopilus* segregate into five distinct clades. The clades have been demarcated following GUZMÁN-DÁVALOS et al. (2003). The sequences of *G. purpureosquamulosus* cluster together in a clade (aeruginosus-luteofolius clade, vide GUZMÁN-DÁVALOS et al. 2003) comprised of species all of those possess a pileus surface covered with reddish to purplish coloured erect squammules towards disc (GUZMÁN-DÁVALOS et al. 2008).

In the clade, the ITS sequence of the Indian collection of *G. purpureosquamulosus* cluster together with other sequences of the same species, collected and subsequently reported from a wide range of phytogeographical zones (GUZMÁN-DÁVALOS et al. 2008) with weak ML bootstrap (50% BS) but with significant posterior probability support (0.95 PP). The Mexican collection of *G. cyanopalmicola* Guzm.-Dáv. cluster with the nrITS sequence of the Indian *G. purpureosquamulosus* with moderate to strong statistical support (63% BS, 0.99 PP) that could be attributed to reason of identical or high similarity of the ITS sequence (GUZMÁN-DÁVALOS et al. 2008).

Based on overall morphology, *G. cyanopalmicola* differs from *G. purpureosquamulosus* by having a stipe surface that turns purple to dark reddish or dark brown when bruised, the absence of pileocystidia, and the presence of considerably larger cheilocystidia (GUZMÁN-DÁVALOS 2006). However, these differences might not be enough to differentiate between these two species as pointed out by GUZMÁN-DÁVALOS (2006). But for the present moment, we believe that more collections of *G. cyanopalmicola* from other regions and additional DNA sequence data are necessary to resolve whether *G. cyanopalmicola* is an independent species or a synonym of *G. purpureosquamulosus*.

The other similar species based on overall morphology, *G. peliolepis* (Speg.) Singer., reported from Brazil, Argentina and Florida (SINGER 1951; HESLER 1969), differs by the presence of pinkish straw-coloured basidiomata with fibrillose scale, smaller basidiospores (6–8 × 4–5.2 µm) with very small warts (almost asperulate) (GUZMÁN-DÁVALOS et al. 2008). *Gymnopilus purpureosquamulosus* can be differentiated from other species with large basidiospores worldwide. *Gymnopilus dilepis* (Berk. & Broome) Singer differs by the presence of the small (10–40 mm) and orange colour of pileus, light

brown spore print, basidiospores with medium-sized to large warts, and very long caulocystidia (18.4–68 × 5.6–14.4 µm) (Guzmen-Dávalos et al. 2003; Thomas et al. 2003). *Gymnopilus purpuratus* differs from *G. pupureosquamulosus* by its bright rust-coloured spore print, weakly dextrinoid basidiospores, and cylindrical to ventricose cheilocystidia *Gymnopilus palmicola* Murrill has considerably larger basidiospores (8–12 × 5.6–7.2 µm) and much larger warts than *G. purpureosquaulosus* (Murrill 1913).

Among previously reported *Gymnopilus* species from India with a yellowish to yellowish brown or orange yellow coloured pileus, *G. pampeanus* (Speg.) Singer differs by its larger pileus, greyish orange spore print, and extended apiculus (0.84–1.69 µm) of basidiospores (Kaur et al. 2014). *Gymnopilus spectabilis* (Weinm.) A.H. Sm differs from *G. purpureosquaulosus* by its orange yellow spore print and very long sterigmata (3.38–6.76 µm) (Smith 1949).

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Authors' contributions: KA identified the species and wrote the text; SP and AKD performed the microscopic work, identified the species, made the phylogenetic analysis, and wrote the text; RS extracted genomic DNA and TS collected the specimen and prepared the macroscopic data.

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